Feedback-generated muscle activity reveals age-dependent decline of motor learning.

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Running title: Motor learning declines with healthy aging

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Number of pages: 41

Number of figures: 5 + 1

Number of tables: 3

Number of words Abstract: 244 words

Number of words Introduction: 632

Number of words Discussion: 1472

Conflict of Interest: The authors declare no competing financial interests.

Acknowledgements:

Funding Sources: NSF BRIGE 1342183, NSF 1535036

Abstract

Recent studies suggest that planned (feedforward-mediated) actions and corrective (feedback-mediated) movements are recalibrated during motor adaptation. However, feedback commands in locomotion are thought to simply reflect sudden environmental changes upon introduction or removal of novel situations independently from sensorimotor recalibration. Thus, we asked if human feedback activity can indicate the motor system's adapted state in walking. We recorded electromyographic (EMG) signals bilaterally on 15 muscles before, during, and after split-belt walking that induces sensorimotor adaptation by moving the legs at different speeds. We exploited the temporal dynamics of feedback commands to isolate them from the overall motor output. We found that EMG aftereffects were dominated by feedback responses, which were structurally different after short vs. long adaptation periods. Only after a long adaptation duration, the structure of each leg's feedback activity resembled the balance-like responses elicited in the contralateral side when the split condition was first introduced. This mirrored feedback activity was a proxy of motor learning at an individual level since it indicated the extent to which each subject adopted the split pattern as their new "reference" gait, such that deviations from it were processed as an opposing perturbation to the one originally experienced. Interestingly, this mirroring decayed with age, but steady state changes during split-belt walking did not, suggesting potentially different neural mechanisms underlying these motor patterns. Taken together our results show that feedback-commands reflect the adapted state of the motor system, which is less flexible as we age.

Significance statement

We showed that feedback activity elicited by sudden environmental transitions is revealing of the underlying recalibration process during sensorimotor adaptation, and hence indicates individual learning capacity. This allowed us to identify age-related decline in motor learning that was not discernible from kinematic measures conventionally used in motor adaptation studies. These findings suggest that older populations may have limited potential to correct their movements through error-based protocols simply given their age. Moreover, we found that split-belt walking recruits distinct motor patterns during and immediately after the split condition, informing our understanding of the therapeutic effect of this task. Therefore, our detailed EMG characterization provided valuable normative data of muscle activity that could be reinforced with repeated exposure of split-belt walking.

1 Introduction

Humans adapt and learn new movements through interactions with the world, but there have been limited efforts investigating the plasticity of muscle activity underlying this motor adaptation. For example, there are several studies in locomotion characterizing changes in kinematics (e.g. Reisman et al. 2005), kinetics (Ogawa et al., 2014; Sombric et al., 2018), metabolic cost (Finley et al., 2013; Sánchez et al., 2017), and the perception of movements (Vazquez et al., 2015) that are retained following walking on a split-belt treadmill that moves people's legs at different speeds. However, none of the work recording muscle activity provides a detailed characterization of what is adapted in muscle space during and after the novel split-belt situation (Ogawa et al., 2014; Raja et al., 2013; Maclellan et al., 2014). It is important to study the adaptation of muscle activity upon sensorimotor learning because the higher dimension of muscles compared to joints allows distinct motor patterns to produce the same movement (Bernstein, 1967). Thus, EMG analysis could reveal distinct neural mechanisms through which motor adaptation occurs that may be obscured by purely assessing changes in kinematic or kinetic variables.

Muscle activity can be adapted through adjusted feedforward- and/or feedback-generated motor commands that contribute distinctively to motor adaptation. Feedforward-generated motor commands are planned ahead of the movement and hence are predictive in nature. Conversely, feedback-generated motor commands arise during movement execution through a transformation of current sensory information into corrective actions (Jordan and Rumelhart, 1992; Bhushan and Shadmehr, 1999). Walking studies presume that only feedforward processes are adapted and underlie the observed aftereffects (Morton and Bastian, 2006; Ogawa et al., 2014). Feedback (a.k.a., reactive) commands are assumed to remain unchanged during locomotor adaptation and simply reflect the walking condition by suddenly changing when the novel environment is introduced or removed (Reisman et al., 2005; Morton and Bastian, 2006; Yokoyama et al., 2018). However, it is unclear whether feedback-generated activity is actually adapted, whether this process is independent of feedforward adaptation, and what impact it has on aftereffects. Dissociating the adaptation (or lack of thereof) feedback and feedforward processes is of interest as they may reflect distinct learning mechanisms (Wagner and Smith, 2008; Yousif and Diedrichsen,

2012).

It is particularly relevant to evaluate the adaptation of feedback and feedforward commands in middle aged and older individuals because of its implications for movement rehabilitation. Namely, locomotor learning induced by split-belt walking can be exploited to improve the gait of stroke survivors (Reisman et al., 2013; Lewek et al., 2017), whose age mostly ranges between 45 and 80+ years old (Mozaffarian et al., 2016). Age-related decline in subjects' ability to adapt movements and the retention of those changes has been previously reported (Wolpe et al., 2016; Sombric et al., 2017)Thus, we studied the plasticity of muscle activity in individuals of age ranges similar to stroke to gain insights on the learning mechanisms available to patients solely because of their age.

In sum, we characterized the adaptation of muscle recordings from a broad set of lower-limb muscles during and after split-belt walking. We contrasted feedback activity to the same environmental transitions, but after distinct adaptation durations to determine if feedback commands mostly indicated changes in the environment or in the motor system's adapted state. We found that the structure of feedback activity revealed subjects' recalibration of walking. Only after a long adaptation duration, feedback activity was mirrored between the legs when compared to the balance-like responses exhibited upon introduction of the split perturbation. This mirroring suggested that individuals adopted the split pattern as their new "reference" gait, such that departures from it were processed as an opposing perturbation to the one originally experienced. Interestingly, older adults showed feedback activity post-adaptation that was less mirrored and more environment-dependent, revealing age-related limitations in sensorimotor learning.

2 Materials and Methods

2.1 Subjects

A group of 16 healthy subjects of ages ranging between 46 to 78 years old (61+-9.9 y.o., 10 female) participated in the study (see Table 2). Their movements and muscle activity were recorded before, during, and after walking on a split-belt. Subject S01 was excluded from our analyses because this individual behaved strangely during the split-belt walking period, which likely compromised this subject's motor adaptation (see Figure 1-1). All subjects provided written informed consent prior to participating in the study, which was approved by the Institutional Review Board at our Institution, and was in accordance to the declaration of Helsinski.

2.2 Experimental Design

We assessed the adaptation and de-adaptation of muscle activity through the protocol illustrated in Figure 1A. The protocol consisted of treadmill walking in six different conditions presented in the following order: Slow Walking (50 strides), Mid Walking (50 strides), Short Exposure (10 strides), Baseline Mid Walking (150 strides), Adaptation (900 strides, in three blocks of 300) and Post-Adaptation (600 strides, in two blocks of 300 or a single 600 block). Slow and mid walking were used to quantify speed-dependent modulation of muscle activity during regular treadmill walking. Short Exposure and Adaptation were used to differentiate learning-dependent changes in muscle activity that required multiple steps in the split environment from those solely due to unexpected introduction or removal of the split perturbation. Baseline walking was used as a reference for steady state walking on the treadmill prior to motor adaptation. Finally, the Post-Adaptation condition was used to evaluate adaptation aftereffects in muscle activity following split-belt walking. The Adaptation and Post-Adaptation conditions were designed to have enough strides to examine the evolution of muscle activity from a transient to a steady state when the perturbation was either introduced or removed, respectively. These conditions were divided into blocks to minimize fatigue and subjects were instructed not to step between blocks to prevent deadaptation due to unrecorded steps.

Belts moved at different speeds (i.e., 'split' mode) only in the Short Exposure and Adaptation conditions with

the dominant leg (self-reported leg used to kick a ball) walking faster. We refer to the dominant leg as the fast leg throughout the text and the non-dominant one as the slow leg. In all other conditions, belts moved at the same speed relative to one another (i.e., 'tied' mode) (Figure 1A). The treadmill was started and stopped at the beginning and at the end of each condition and speeds did not change while each condition was ongoing. Belts moved at one of three possible speeds throughout the experiment: a self-selected ('mid') walking speed, a 'slow' speed of 66.6% of the mid speed, and a 'fast' speed of 133.3% of the mid speed. We used self-selected speeds to ensure subjects from all ages could complete the entire protocol. The self-selected speed was obtained by first averaging each subject's speed when walking over ground in a 50-meter hallway during 6 minutes (i.e., 6-min walking test (Rikli and Jones, 1998)) and then subtracting $0.35m/s$, which resulted in a comfortable walking speed on a treadmill based on pilot data in older adults ($>$ 65 yrs). Mid speed was $0.72 \pm 0.26m/s$ (mean $\pm$ standard deviation) across the population. Speed values used for all subjects during baseline walking are shown in Table 2.

Safety measures were designed such that participants from older ages could complete the study. First, all subjects wore a harness that only provided weight support in the event of falling but not during walking or standing. Also, subjects were told a few seconds in advance that they could hold on to a handrail (directly located in front of them) whenever a condition or block started or finished. Subjects were encouraged to let go of the handrail as soon as they felt comfortable doing so to minimize the effect of external support on muscle recordings. Finally, we monitored subjects' heart-rate continuously and blood-pressure during the rest breaks to prevent over exertion in any of the participants.

2.3 Data acquisition & analysis

Data collection

We collected electromyographic (EMG) signals, kinematics, and kinetic data to characterize subjects behavior. Surface EMG signals from 15 muscles on each leg were recorded for all subjects (see Table 1 for full list and abbreviations) at 2000Hz using a Delsys Trigno System (Delsys Inc., Natick, Massachussets). Signals were

highpass-filtered to remove undesired movement artifacts and then rectified. We used a 2nd order Butterworth filter (dual-pass) with a cutoff frequency of 30Hz, which resulted in 80 dB/dec attenuation and zero-lag (Merletti and Parker, 2004). Unlike other studies (e.g., Torres-Oviedo and Ting 2007), we did not apply a subsequent lowpass filter following rectification as we did not require the EMG envelope for our analysis (see below). Kinematic data was collected at 100 Hz with a passive motion analysis system (Vicon Motion Systems, Oxford, UK). Movements were recorded by placing reflective markers bilaterally on bony landmarks at the ankle (i.e., lateral malleolus) and the hip (i.e., greater trochanter). Ground reaction forces were recorded with an instrumented split-belt treadmill (Bertec Corporation, Columbus, Ohio) and sampled at 1000Hz. Forces along the axis of gravity (Fz) were used to determine when the foot landed (i.e., heel-strike: $Fz > 10N$) or was lifted off the ground (i.e., toe-off: $Fz < 10N$).

Data Analysis

EMG parameters: We characterized the changes in EMG during and after split-belt walking by first generating muscle activity variables that discretized each muscle's activity over the gait cycle (Figure 1B). Filtered EMG activity was divided in sub-intervals of the gait cycle aligned to gait events to focus on changes in muscle activity within the gait cycle, rather than on activation changes due to differences in timing of the gait cycle across the distinct walking conditions (Dietz et al., 1994; Reisman et al., 2005). For example, in Figure 1B we present sample EMG traces for Baseline walking at medium speed (gray trace) and late Adaptation (green traces) for the leg walking slow (top panel) and the one walking fast (bottom panel). More specifically, we divided the gait cycle of each leg into 6 intervals according to well defined gait phases (Perry and Burnfield, 2010): first double-support (from ipsilateral heel-strike to contralateral toe-off), early single-stance (first half of single stance), late single-stance (second half of single stance), second double support (from contralateral heel-strike to ipsilateral toe-off), early swing (first half of swing phase) and late swing (second half of swing phase). In order to achieve better temporal resolution, each of these 6 intervals were further divided into two equal sub-intervals, yielding 12 intervals for each gait cycle. Note that the normalized gait cycles appear to be the same duration because of our normalization procedure. However, each of these sub-intervals' duration

ranged between 75 and 120 ms. The precise timing for each sub-interval throughout the different epochs of the study are presented in Table 3. Muscle activity amplitude was averaged in time for each of these sub-intervals, for every stride and muscle resulting in 360 muscle activity variables per subject: 12 intervals x 30 muscles. Sample of these variables for one muscle in one gait cycle for Baseline (B_{raw}) (gray row) and late Adaptation ($LateA_{raw}$) (green row) are presented in the rows below the EMG traces in Figure 1B.

These EMG variables were normalized using the activity during Baseline walking as a reference. To this end, we first computed the mean activity for each sub-interval across the last 40 strides (i.e., steady state) of Baseline walking. Then each muscle's activity was scaled such that the least and most active phases for every muscle during baseline had the value of 0 and 1, respectively. We specifically subtracted the mean value for the least active phase in Baseline from the activity in all sub-intervals for all strides in all conditions and we subsequently divided the shifted values by the mean baseline value for the most active phase. Following this normalization, the units of EMG activity for each muscle of a given subject were represented with respect to the average maximum value recorded in that muscle during Baseline walking of the same subject. This scaling allowed us to aggregate subjects and compare effect sizes across muscles even when recorded EMG amplitudes were very different because of sensor placement or underlying tissue properties (Figure 1D).

Kinematic parameters: The adaptation of movements was characterized with step-length asymmetry, which is a metric known to change during and after split-belt walking (Reisman et al., 2005). We computed step-length asymmetry on each stride cycle by calculating the difference in step lengths (i.e., ankle to ankle distance at foot landing) for two consecutive steps taken with the fast and slow leg. This difference was normalized by the sum of step lengths to obtain a measure that was a proportion of each subjects' step sizes. A zero step-length asymmetry value indicated that steps lengths were even, negative values indicated that the (non-dominant) leg walking on the slow belt was taking longer steps than the (dominant) one on the fast belt and viceversa for positive values.

We also computed body displacement with respect to the foot in contact with the ground during the stance phase for each leg. This was done to interpret the changes in muscle activity upon transitions between tied and split

conditions. Body displacement was computed as the anterior-posterior distance between the middle of the hip markers (greater trochanter) and the ankle from ipsilateral heel-strike to contra-lateral heel-strike. To focus on displacement changes within this period of the stance phase, we aligned and divided the body displacement in two sub-intervals: double support (from ipsilateral heel-strike to contra-lateral toe off) and single stance (from contra-lateral toe-off to contra-lateral heel-strike). This normalization of the time axis facilitated the comparison of body displacement during stance of the fast vs. the slow leg or across walking conditions given the distinct stance durations between legs and treadmill modes (tied vs. split) (Reisman et al., 2005).

Outcome measures: EMG and kinematic parameters were used to characterize the behavior during six experimental epochs (Figure 1): Short Exposure (all strides except first and last), early and late Baseline, early and late Adaptation, and early Post-Adaptation. 'Early' epochs were characterized by the average of the initial 15 strides and 'late' epochs by the average of the last 40 strides for each condition. Recordings during the very first and last stride of each condition were excluded to eliminate from the analysis effects linked to the treadmill's start and stop. For example, early Adaptation consisted of the mean activity for strides 2-16, such that 15 strides were considered but the first one was excluded.

We compared muscle activity for a given epoch with that of Baseline walking (Figure 1D). This allowed us to characterize how motor commands differed from normal walking along the adaptation process. The comparison was done by calculating differences in muscle activation variables between an epoch of interest (e.g. $LateA_{raw}$) and those during Baseline (B_{raw}) walking (e.g., $EarlyA = EarlyA_{raw} - B_{raw}$). We also computed differences in muscle activation variables between consecutive epochs in which subjects transitioned from the split-to-tied (e.g., $FBK_{split-to-tied} = EarlyP - LateA$) or tied-to-split conditions (e.g., $FBK_{tied-to-split} = EarlyA - B = EarlyA$). The magnitude of these was calculated by computing the euclidean norm of the 360-dimensional vectors (e.g., $\|FBK_{tied-to-split}\|$). This offered a quantification of overall changes in muscle activity with one single metric, which was particularly relevant for $EarlyP$ since it indicated the aftereffect size in muscle space.

2.4 Characterizing feedback components of muscle activity

Previous studies have dissociated the adaptation of feedforward and feedback commands through restriction of limb trajectories when error signals are known (Scheidt et al., 2000; Wagner and Smith, 2008; Albert and Shadmehr, 2016), which is not the case for walking. Thus, we propose to dissociate the adaptation of feedforward and feedback components of muscle activity through an operational definition that exploits the distinct temporal dynamics of these processes. We specifically assume that feedforward components change slowly upon experiencing a novel situation because they are generated purely through internal models that are updated gradually. On the other hand, feedback components can change rapidly after a transition in the walking environment since sensory information is available immediately following a change in the walking context. Note that our definition of feedback-generated motor commands includes modulation of muscle activity that occurs at different latencies after a movement disturbance (i.e., short- and long-latency reflex responses and subsequent voluntary responses) (Horak et al., 1990). In sum, we quantified feedback-generated activity as the changes in EMG after a sudden environmental transition (i.e., EMG_{after}) with respect to EMG activity before the transition (i.e., EMG_{before}). In equation form:

$$EMG = FF + FBK$$

Where EMG is the measured muscle activity whereas FF and FBK are the feedforward- and feedback-generated muscle activity, respectively. We assume that feedforward components change slowly upon sudden transitions:

$$\Delta FF \approx 0$$

Thus:

$$\Delta EMG = EMG_{after} - EMG_{before} = \Delta FBK + \Delta FF \approx \Delta FBK = \text{FEEDBACK-GENERATED ACTIVITY}$$

Therefore, even if the change in EMG is strictly equal to the change in both feedback- and feedforward-generated activity, we approximated the latter one to be negligible because it occurs at a slower rate compared to changes in feedback-generated activity after an abrupt transition in the walking condition. For simplicity, we refer to ΔFBK as FBK. For example, in the split-to-tied transition this results in:

$$FBK_{split-to-tied} = EarlyP - LateA \tag{1}$$

Where $LateA$ and $EarlyP$ are the unbiased muscle activity before and after the split-to-tied transition, respectively. This equation simply formalizes the idea that feedback-generated activity might change immediately after removing the split-belts environment. Importantly, the muscle activity aftereffects ($EarlyP$) are not the same as our definition of the feedback-generated activity upon removal of the split condition (Eq. 1), but contribute to it. Namely, we anticipate that aftereffects will be composed of the learned motor pattern carried over from late Adaptation to Postadaptation (i.e., feedforward component) plus a feedback component due to the sudden environmental transition.

We analyzed the structure of the feedback component of aftereffects ($FBK_{split-to-tied}$) to gain insight into learning-dependent mechanisms. More specifically, we considered three possibilities for the feedback component of aftereffects in muscle space (Figure 1C): 1) negligible relative to feedforward commands (C1), 2) solely environment-dependent (non-adaptive) feedback responses (C2), and 3) adaptive feedback responses (C3). The basis and rationale for each one of these possibilities is presented in the following sections.

2.4.1 Case 1 (C1): Feedback contribution is negligible compared to feedforward contribution to after-effects

One possibility is that muscle activity is mostly controlled in a feedforward manner when the split perturbation is removed. In other words, feedback responses are small in magnitude when compared to the feedforward components. Thus, muscle activity before ($LateA$) and after ($EarlyP$) the split-to-tied transition are roughly the same, as illustrated in the schematic of c1 in Figure 1C. This possibility suggests that feedback components are present during the tied-to-split transition ($EarlyA$), but are greatly reduced during the split-to-tied transition because the return to a familiar environment (i.e., tied condition) may not require the same extent of feedback responses as when a novel condition (i.e., split) is experienced. We formulated this case on the basis that aftereffects result from the continuation of the motor output updated during the Adaptation period (Malone et al., 2012). Formally expressed:

$$FBK_{split-to-tied} = EarlyP - LateA = 0 \;\Rightarrow\; EarlyP = LateA$$

2.4.2 Case 2 (C2): Feedback-generated motor commands are environment-dependent

Another possibility is that feedback responses are not negligible during post-adaptation, but they are not adaptive in nature. That is, the feedback processes generating them are not updated during split-belt walking and produce corrective muscle patterns solely as a function of the sensed environment. There are two expected observations if feedback motor commands are purely environment-dependent. First, feedback-generated activity would change similarly after removing the split perturbation following a long or short exposure to the split condition because the environment transitions identically in these two situations. Second, we would expect that changes in muscle activity originated by the tied-to-split transition (i.e., $FBK_{tied-to-split}$) would be numerically opposite from those from the split-to-tied transition (Figure 1C, c2 schematic). In other words, any muscle whose activity increased when the split perturbation was introduced, should decrease by the same amount when it is removed (and vice versa). We reasoned that this feedback case would be plausible given that split and tied walking require distinct motor patterns and subjects might switch between them. Thus, the immediate changes in activity would be numerically opposite, as expected when switching back and forth between two motor patterns (A to B vs. B to A). Formally expressed:

$$FBK_{split-to-tied} = EarlyP - LateA = -EarlyA \;\; \Rightarrow \;\; EarlyP = LateA - EarlyA$$

Where $EarlyA$ represents muscle activity during the Early Adaptation with respect to Baseline.

2.4.3 Case 3 (C3): Feedback responses indicate the motor system's sensorimotor recalibration

Finally, we considered the possibility that substantial feedback responses are not only present following transitions in walking speeds, but they are indicative of the motor system's recalibration during sensorimotor adaptation. In this case, changes in activity from the split-to-tied conditions would be different after a short vs. a long exposure to the split environment. We specifically considered that following the long exposure, the motor system would learn that the split (perturbed) condition is the "new normal". Consequently, removal of the split condition would be processed as the opposite perturbation to the one experienced when the novel environment was first introduced. We based this possibility on previous work reporting that the removal of the novel condition

is in-and-of itself a perturbation (Herzfeld et al., 2014b), and that feedback responses are adapted through experience to an altered environment even if there is no feedback-specific learning opportunities (Wagner and Smith, 2008; Yousif and Diedrichsen, 2012). During the split-to-tied transition in our experiment the leg that was 'fast' moves down in speed, while the one that was 'slow' moves up. This is the mirrored version of the initial tied-to-split transition where the 'fast' leg went up and the 'slow' leg went down. Accordingly, we expect the feedback-generated muscle activity to reflect this mirror-symmetry between belt-speed transitions when the split condition is introduced or removed. Formally expressed:

$$FBK_{split-to-tied} = EarlyP - LateA = EarlyA^* \;\; \Rightarrow \;\; EarlyP = LateA + EarlyA^*$$

Where $EarlyA^*$ is $EarlyA$ mirrored (i.e., transposing the values for the left and right legs).

2.5 A general regression model to characterize the contribution of feedback-generated activity to aftereffects in muscle activity

In summary, we tested the extent to which each one of these three possibilities shaped the structure of feedback-generated components of aftereffects with one single regression model:

$$FBK_{split-to-tied} = EarlyP - LateA = -\beta_S EarlyA + \beta_M EarlyA^* \tag{2}$$

Where the parameters β_S and β_M are respectively interpreted as the extent of feedback-generated activity that is solely environment-dependent (β_S) or adaptive (β_M). Thus, the three cases presented in the previous section can be summarized through the β coefficients as: (C1) negligible feedback contributions to aftereffects in muscle space ($\beta_S = \beta_M = 0$), (C2) solely environment-dependent (non-adaptive) feedback responses ($\beta_M = 0$ and $\beta_S = 1$), and (C3) adaptive feedback responses ($\beta_M = 1$ and $\beta_S = 0$). Note that *beta* coefficients might be smaller than expected even if the structure of feedback muscle activity is predicted by (C2) or (C3) because magnitude differences between $FBK_{split-to-tied}$ and $EarlyA$. To dissociate the effect of magnitude from that of structure of feedback responses, we performed a secondary analysis in which we tested the alignment of the $FBK_{split-to-tied}$ vector and the regression vectors $-EarlyA$ and $EarlyA^*$ by computing the cosine of the angles they form. Under (C2) we expect $FBK_{split-to-tied}$ to be aligned to $-EarlyA$ (cosine of 1), and under (C3) we expect it to be aligned to $EarlyA^*$. All analyses were performed for both individual and group averaged

patterns of activity. It is worth pointing out that our regression analyses rely on the assumption that initial muscle responses during early adaptation $EarlyA$ are not anti-symmetric (i.e., same group of muscles increasing in a leg, decrease in the other one). Under this assumption, the mirrored $EarlyA^*$ and opposite $-EarlyA$ muscle vectors are different from each other in muscle space (i.e., not colinear). This assumption was confirmed empirically, as the cosine of the angle formed by the two vectors was -0.13 ± 0.38 (median $\pm$ inter-quartile range across subjects).

2.6 Statistical analysis

Differences in muscle activity across epochs. Significant changes in EMG variables between any pair of epochs were determined by using the Wilcoxon signed-rank test (i.e., non-parametric analogue of a paired t-test) on each of the 360 EMG parameters. Effect sizes were computed using median values across subjects since this is a measure less susceptible to outliers. All tests were two-tailed and the null hypothesis was that the (normalized) absolute effect size was smaller than 0.1 (i.e., 10% of the maximum baseline activity for that muscle). This was done to avoid finding significant but small, and presumably meaningless, differences. We corrected for multiple comparisons using the Benjamini-Hochberg procedure to control for False Discovery Rate (FDR) (Benjamini and Hochberg, 1995; Kass et al., 2016) for which we set an acceptable FDR to be 10%. We reasoned that this FDR value was adequate given our conservative approach of only considering significant effect sizes > 0.1. Threshold p-values from the FDR procedure are reported for each application.

Structure of feedback activity. The linear regressions characterizing the structure of feedback-generated activity were performed using Matlab's *fitlm()* function and computing (Pearson's) R^2 values that were uncentered, given that our regression model did not include intercept terms. We compared the regressors obtained for data following the Short Exposure and Adaptation epochs using a two-tailed paired t-test. We report p-values, as well as mean changes and well as Cohen's d for effect size.

Analysis of inter-subject variability. We conducted post-hoc regression analyses to determine if either age or

walking speed could explain the large inter-subject variability that we observed in the regression coefficients. We focused on these subject-specific features because they exhibited large ranges in our cohort that could have impacted our results. We also studied the association between aftereffects sizes in muscle activity and step-length asymmetry. For all these analyses we applied Spearman's correlations as a non-parametric alternative to Pearson's correlation because it is more robust to potential outliers. The correlation value (lowercase r, to distinguish from previous use of R) and the corresponding p-value were presented.

Data and code availability: all data and code used for this study are available at `https://figshare.com/account/home#/projects/28071`.

3 Results

3.1 Muscle activity during early Adaptation reflects feedback control

Muscle activity when the split condition was introduced primarily reflected feedback-generated motor commands. This is shown by the changes in body position Figure 2A and muscle activity Figure 2C during early Adaptation compared to Baseline walking (i.e., *EarlyA*). We observed that the body position (i.e., hip position) with respect to the standing foot during stance phase (e.g., fast leg's foot for the fast stance) was either farther in front (positive values) during fast stance or farther back (negative values) during slow stance in early Adaptation compared to Baseline walking (Figure 2A). In other words, subjects were either "falling" forward or backward during early Adaptation immediately after hitting the ground (heel-strike) with the fast or slow legs, respectively. This was likely due to the discrepancy between subjects' expected and actual walking speed (Figure 2B). Consistently, the corresponding muscle activity for both legs during 100-400ms (colored rectangles) after heel-strike (onset of movement perturbation at each stride) resembled those of feedback-mediated postural responses over comparable time windows (Torres-Oviedo and Ting, 2007; Chvatal and Ting, 2012; Safavynia and Ting, 2012; de Kam et al., 2017). Namely, the standing fast leg following fast heel-strike (blue rectangle) initially increased the activity of posterior muscles in the ankle (MG, LG, SOL) and knee (SMB, SMT, BF) followed by increased activity in quads (VM,VL,RF) and hip flexors (ADM,HIP, TFL) (Figure 2C), which has been previously observed when the body falls forward (Chvatal et al., 2011; Chvatal and Ting, 2012). Concurrently, the slow leg exhibited greater activity of ankle (TA) and hip flexors (RF, ADM, HIP) to presumably swing the leg more vigorously as a possible feedback-mediated stepping balance response (Horak and Macpherson, 1996). Similarly, the standing slow leg following the slow heel-strike (orange rectangle) initially increased the activity of anterior muscles (TA, quads -RF, VM, and VL-, and hip flexors -HIP,ADM,TFL-) and reduced the activity of calf muscles (MG, LG, SOL) (Figure 2C) as has been observed in balance responses when the body falls backward (Tang and Woollacott, 1999; Chvatal et al., 2011; Chvatal and Ting, 2012). On the other hand, some activity during early Adaptation might have been modulated according to motor demands that persist throughout the split-belt condition. Particularly, the fast leg's swing activity (increased TA followed by reduced hamstrings -SMB, SMT, and BF-) and the activity of ankle muscles (MG, LG, SOL, PER) during later phase of stance for both legs were also observed during late Adaptation (Figure3B). In sum, most of the

changes observed during early Adaptation matched those of feedback-mediated responses to counteract balance perturbations induced by the novel environment.

3.2 Anti-symmetric modulation of muscle activity during late Adaptation is not a direct reflection of each leg's walking speed

Muscle activity during late Adaptation was not simply regulated as a function of the speed at which each leg moved in the split condition. Overall we observed an anti-symmetric modulation of muscle activity of the two legs during late Adaptation with respect to Baseline (*LateA* displayed in Figure 3B). Namely, we found that if a group of muscles increased activity on one side, the same group decreased activity on the other one. Interestingly, this opposing modulation across legs was not merely determined by ipsilateral walking speed. Should this have been the case, one would expect reduced muscle activity of the slow leg with respect to mid-speed Baseline as seen in slow walking (Figure 3A) and increased on the fast leg (Den Otter et al., 2004; Dietz et al., 1994). However, this type of modulation was only observed in distal muscles (MG, LG, SOL, PER, TA) and in the fast leg's hip flexors (HIP, TFL, ADM), but not in most of the proximal muscles (SMB, SMT, BF, RF, VM, VL, ADM, TFL). In fact, proximal muscles crossing the knee (SMB, SMT, BF, RF, VM, VL) and the slow leg's hip (ADM, TFL) increased in the leg walking slow and decreased in the leg walking fast. This suggested that their activity was predominantly regulated by the speed of the contralateral side. This was least anticipated in quads (RF, VM, VL) and hip flexors (ADM, TFL) of the standing leg since their activation was opposite to that of distal muscles (MG, LG, SOL) within the same leg. In contrast, the modulation of hamstrings (SMB, SMT, BF) during swing was less unexpected since it matched concurrent modulations of the contralateral standing leg's calf muscles. In sum, muscle activity for split-belt walking highlights the interlimb nature of locomotion because it exhibited an interaction between muscle group (i.e., distal vs. proximal) and walking speed, rather than individual leg modulation based on speed.

3.3 Feedback responses are mirrored between the legs when returning to tied-belts after prolonged exposure to split-belt walking

We proposed that aftereffects ($EarlyP$) would be generated by a combination of feedforward and feedback processes. In particular, we considered the possibility that feedforward-generated motor commands would dominate EMG aftereffects (C1), such that muscle activity during Early Post-Adaptation would be similar to that of Late Adaptation ($LateA$). Qualitatively, we found little resemblance in the muscle activity during these two epochs, as shown by the few significant changes that were similar between them (black outlines in Figure 3B and 3C). This indicated that feedback-generated motor commands also contributed to muscle patterns during Early Post-Adaptation. To quantitatively characterize the structure of feedback activity, we used a regression model ($FBK_{split-to-tied} = -\beta_S EarlyA + \beta_M EarlyA^*$) that contrasted feedback-generated activity upon introduction ($FBK_{tied-to-split}$) and removal ($FBK_{split-to-tied}$) of the split perturbation (Figure4). Recall that the purely environment-dependent feedback responses (C2) indicated that muscle patterns upon introduction and removal of the split condition would be numerically opposite (i.e., $FBK_{split-to-tied} = -\beta_S EarlyA$, with $\beta_S = 1$), whereas the adaptive possibility (C3) indicated they would mirror each other (i.e., $FBK_{split-to-tied} = \beta_M EarlyA^*$, with $\beta_M = 1$). Note that $EarlyA^*$ is the mirrored version of $EarlyA$, such that the slow leg's activity in one transition would exactly be the fast leg's activity in the other transition and vice versa. Lastly, C1 suggested that feedback-mediated activity would be negligible compared to feedforward one (i.e., $\beta_S = 0$ and $\beta_M = 0$).

Our regression analyses were consistent with mirrored feedback activity (C3) when using both group averaged activity (Figure 4E, magenta dot: CI for $\beta_M = [.554, .657]$, $\beta_S = [.144, .246]$, $R^2 = 0.648$), and muscle activity for each individual (Figure 5A, small magenta dots; median $\pm$ inter-quartile range for $\beta_M = 0.427 \pm 0.113$, $\beta_S = 0.13 \pm 0.289$, and $R^2 = 0.309 \pm 0.187$). The mirroring of activity appears to be less than expected under C3 (i.e., $\beta_M < 1$ in all individuals and group averaged data), which could be simply an underestimation of actual mirroring due to differences in magnitude between $FBK_{split-to-tied}$ and $FBK_{tied-to-split}$. In fact, $FBK_{split-to-tied}$ magnitude was on average 88.9% of the $FBK_{tied-to-split}$ magnitude for individual subjects and 79.9% on group median data. To account for this effect, we computed the cosine of the angle between $FBK_{split-to-tied}$ and the regression factors $EarlyA^*$ and $-EarlyA$ to compare the alignment of feedback activity to the other two vectors. We found that $FBK_{split-to-tied}$ and $EarlyA^*$ were more aligned when using group

median activity (cosine of 0.778) and individual data (median $\pm$ inter-quartile range: 0.528 ± 0.232) than $FBK_{split-to-tied}$ and $-EarlyA$ for group (cosine of 0.367) and individual data (median $\pm$ inter-quartile range: 0.18 ± 0.556). There are other methodological factors that limited our assessment of mirroring. First, consider that any measure of mirroring requires expressing the activity of one leg as a function of the other. Thus, β_M (but not β_S) and vector alignment can be biased downwards by any asymmetries between the legs caused by mismatched placement of the EMG sensors or temporally misaligned activity caused by unequal half-cycle durations. Second, any noise in the regression factors (i.e., $EarlyA$ and $FBK_{split-to-tied}$) biases the estimates of the regressors towards 0. This is because noise in a high-dimensional space is more likely to lead to the misalignment of the vectors. Both of these problems are made more acute for individual regressions, which have larger noise levels, and where activity is more likely to be asymmetrically recorded. Consistently, the effect of noise can be reduced by using a larger number of strides to characterize muscle activity during 'early' epochs. For example, similar results were obtained when using 15 strides, rather than 5, but with slightly larger regression coefficients and R^2 (e.g., group averaged activity with 15 strides: CI $\beta_M = [.588, .695]$, $\beta_S = [.222, .329]$, $R^2 = 0.665$). Despite these methodological limitations, the regression and cosine-based analysis indicated that feedback activity in the split-to-tied transition was substantially more similar to the mirrored (C3) than then numerically opposite (C2) feedback activity in the tied-to-split transition.

Our regression and cosine-based results are qualitatively supported by the remarkable similarity between the slow leg's pattern at tied-to-split transition (Fig. 4C, top half) and the one of the fast leg at split-to-tied transition (Fig. 4D, bottom half). This mirroring was also observed when comparing the contralateral sides (Fig. 4C bottom half to 4D top half) at these transitions, except for the reduced activity in muscles RF, VL, VM, GLU, TFL during early stance only observed in $FBK_{split-to-tied}$ (Fig. 4D). We also found that the body motion exhibited mirroring between the legs' stance phases. For example, the change in body position with respect to the fast stance leg in the tied-to-split transition (Fig. 4F, green trace) closely resembled the one during slow stance at the split-to-tied transition (magenta trace). Thus, both EMG and kinematics exhibited mirroring when we consider changes with respect to the previous steady-state condition. Further, note that these body changes were distinct from the actual location of the body with respect to baseline walking illustrated in gray. In sum, these results suggest that the nervous system (specifically, the feedback response mechanisms) adopted the

steady-state behavior as a new reference gait pattern. Therefore, kinematic perturbations away from this new reference, rather than perturbations away from the subjects' baseline behavior may drive feedback-generated muscle activity.

Lastly, to confirm that the mirroring between legs is a learning-dependent process, and not simply due to removal of the split perturbation, we ran the same regressions on the split-to-tied transition following the Short Exposure condition (i.e., we used the exact same regression factors as in the analysis for the (long) Adaptation period). In this condition subjects did not have time to adapt, so we expected to observe solely environment-dependent changes in EMG activity (C2), rather than mirroring (C3). Indeed, that is what we found with our regression model applied to the transition after the Short Exposure. This was true for both the group-averaged activity (Figure 4E, gray dot; CI for $\beta_M = [-.148, .039]$, $\beta_S = [1.1, 1.21]$, $R^2 = 0.829$) and the activity of individual subjects (data not shown) (median $\pm$ inter-quartile range for $\beta_M = 0.081 \pm 0.297$, for $\beta_S = 0.728 \pm 0.442$, and for $R^2 = 0.469 \pm 0.171$). In addition, our cosine-based analysis returned values of 0.169 and 0.014 ± 0.204 for the group and individual vectors to $EarlyA^*$ respectively, and 0.917 and 0.668 ± 0.139 for vectors to $-EarlyA$. In sum we found a strong dissociation of the structure of change in feedback responses and exposure duration: following the Short Exposure condition, feedback muscle activity can be modeled as environment-dependent, whereas following Adaptation (long exposure) the feedback is better explained by mirroring. Namely, comparison of the individual coefficients indicated that every subject has a higher β_M and smaller β_S after the long than the short exposure, with a median change of 0.41 and -0.649 and Cohen's d of 1.61 and -2.72, respectively (long vs. short: β_M: $p = 3.03 \times 10^{-6}$ and β_S: $p = 3.56 \times 10^{-10}$). Consistently, every subject's feedback responses were more aligned to $EarlyA^*$ and less to $-EarlyA$ following the long exposure compared to the short one, with a mean cosine change of 0.563 and -0.543, and corresponding Cohen's d of 1.94 and -2.82 respectively (long vs. short: $p = 2.75 \times 10^{-6}$ and $p = 3.05 \times 10^{-8}$). Taken together, we found that feedback activity contributed to aftereffects in muscle space and its structure reflected the recalibration of the motor system during adaptation.

3.4 Healthy aging reduces the adaptation of feedback-generated muscle activity contributing to aftereffects

Analysis of intersubject variability revealed that older adults exhibited less adaptation of feedback-generated activity. Namely, β_M and β_S were associated to subjects' age ($r = -0.63$, $p = 0.014$ and $r = 0.69$, $p = 0.0058$ respectively, Figure 5A) with older subjects showing smaller β_M and larger β_S. This indicates that feedback processes in older adults was less adapted (and more environment-driven) compared to younger adults. We also noticed that the magnitude of feedback activity were possibly smaller in older adults ($\|FBK_{split-to-tied}\|$: $r = -0.69$, $p = 0.0055$; $\|FBK_{tied-to-split}\|$: $r = -0.52$, $p = 0.049$; Figure 5B). These smaller feedback responses in older individuals could make it more difficult to identify the structure of feedback activity because of the reduced signal to noise ratio, leading to biased or noisy estimates of β_M and β_S. To discard this possibility, we correlated the R^2 of the fitted models with age. We found no effects of age ($r = -0.43$, $p = 0.11$, not shown), meaning that the regression model applied to individual data captured comparable levels of variance regardless of subject age. In other words, while we observed variability in the model fits across individuals (as indicated by the R^2 range), the aging-dependent effect was not driven by the inter-subject differences in the quality of regression fits.

Taken together, we observed an actual age-mediated difference in the extent of mirroring of feedback muscle activity, suggesting an age-mediated decline of learning processes updating feedback responses. This limited learning capacity in older individuals was further supported by the negative association between age and EMG aftereffects ($\|EarlyP\|$, $r = -0.82$, $p = 0.00026$ Figure 5E): the older the subjects, the smaller the aftereffects. This happened despite the similar magnitude of muscle activity during late Adaptation across individuals ($\|LateA\|$ vs. age: $r = 0.064$, $p = 0.82$, Figure 5C). Importantly, walking speed, which naturally alters muscle activity, was not associated to either the magnitude of EMG aftereffects ($p = 0.11$) nor to the adaptation (speed vs. β_M $p = 0.75$) or magnitude of split-to-tied feedback responses ($p = 0.53$). While subjects walking faster experienced larger speed differences (i.e., larger perturbations), we believe that the individual perturbation's magnitude were not distinct enough across subjects to have an effect on motor learning (i.e., EMG aftereffects and β_M). Interestingly, age-dependency was not observed in the magnitude of step-length asymmetry aftereffects ($r = -0.34$, $p = 0.21$, Figure 5D), which are conventionally used to characterize locomotor

learning. This suggests that the adaptation of muscle activity may be more sensitive to individual differences in learning than kinematics. Alternatively, there might be partially different mechanisms involved in the adaptation of muscle activity and step length asymmetry. In agreement, muscle activity aftereffects and kinematic aftereffects were not correlated ($r = 0.346$, $p = 0.206$, not shown). Based on these latter regression results one could consider that there are two different learning mechanisms: one that underlies the association between age and EMG aftereffects, and a separate one captured by kinematics aftereffects. In sum, we observed diminished mirroring of feedback activity post-adaptation and smaller EMG aftereffects in older adults, suggesting that healthy aging has a negative impact on learning process updating feedback-generated muscle activity.

4 Discussion

We showed that EMG aftereffects were not merely a continuation of motor patterns learned during split-belt walking, but exhibited a large contribution of feedback-generated motor commands. Importantly, the structure of feedback activity post-adaptation resembled balance-like responses that were mirrored between the legs when compared to those elicited in response to the split perturbation. This mirrored feedback activity indicated that departures from an updated "reference" gait induced corrective responses similar to those when a novel environment was introduced. Therefore, we interpret the mirrored feedback commands as a proxy of motor learning since they were only observed when subjects had enough time to adopt the split pattern as their new reference gait. Interestingly, older subjects showed less mirrored feedback activity between the legs leading to smaller aftereffects in muscle space, and thus revealed less adaptation of walking. Our results are relevant not only because we identified a sensitive measure of individual motor learning, but also because we provided a valuable characterization of normative changes in muscle activity from split-belt walking, which has been suggested as a rehabilitation strategy.

EMG aftereffects are not a continuation of muscle activity during late adaptation. We found different muscle activation patterns during and after split-belt walking. Muscle activity during split-belt walking were mostly anti-symmetric, likely due to the asymmetric demands of the split environment, such as the distinct speed-specific propulsion force demands for each leg (Sombric et al., 2018). However, motor patterns were not simply adjusted based on ipsilateral speed demands, but activity of proximal muscles seemed influenced by the speed of the other side. This highlighted the bilateral nature of locomotion (Maclellan et al., 2014) and contrasted with the idea that each leg is adapted independently, as observed in hybrid split-belt walking (i.e., one leg moving forward fast and the other leg moving backwards slow) (Choi and Bastian, 2007) possibly because of the peculiar demands of this task. The learned anti-symmetric pattern only partially resembled the initial feedback responses of a few muscles, providing limited evidence for feedback activity leading the feedforward pattern of the new environment (Thoroughman and Shadmehr, 1999; Albert and Shadmehr, 2016). In fact, most muscles increased activity when the split condition was introduced and plateaued at reduced activation levels, supporting the notion that initial stability demands result in co-activation (Franklin et al., 2008) that decreases as motor patterns become more efficient (Huang and Ahmed, 2014; Finley et al., 2013).

The learned (steady-state) pattern continued in only a few muscles when the split condition was removed, which was to a certain degree unexpected given that kinematic aftereffects are thought to reflect a continuation of the adapted motor commands in the altered environment (Malone et al., 2012; Morton and Bastian, 2006; Ogawa et al., 2014). Plantarflexors (calf muscles) were the predominant muscles exhibiting similar activity before and after split-belt walking (only during double support). Plantarflexor activity contributes to displacing the body and leg forward (Neptune et al., 2001), and consequently modulate step length (Neptune et al., 2008). Thus, continuation of plantarflexors' anti-asymmetric activity post-adaptation might lead to the step length asymmetry following split-belt walking. To test this hypothesis, future work is needed with a different approach, such as musculoskeletal simulations (Steele et al., 2010; Song and Geyer, 2015), which will enable investigating the EMG-kinematic relation during and after split-belt walking. In sum, muscle activity in the split condition was anti-symmetric and only plantarflexors' activity during late adaptation continued upon removal of the split environment.

Feedback activity post-adaptation indicated the motor system's adapted state We demonstrated that the structure of feedback activity post-adaptation indicated subjects' adapted state and not just environmental changes. This was shown by the difference in feedback activity after identical environmental transitions following distinct exposures to the split condition. Feedback activity was only mirrored after a long adaptation period because subjects require several steps in the novel environment before updating their representation of the world (Roemmich and Bastian, 2015) and adopting the novel situation as the new "normal". The mirrored feedback activity also predicts that muscle patterns induced by removal of a perturbation would be similar to those elicited by introduction of the opposite perturbation, which would facilitate meta-learning (Herzfeld et al., 2014b). The notion that feedback responses are influenced by the adaptation process is in accordance with previous reaching studies (Wagner and Smith, 2008; Crevecoeur and Scott, 2013; Cluff and Scott, 2013). We consider three possibilities on how the feedback loop generating feedback responses may be adapted. First, feedback adaptation might be merely a byproduct of feedforward adaptation. That is, feedback activity post-adaptation might emerge in response to unintended consequences from executing updated motor plans (feedforward) that are inappropriate for the current environment. While possible, this option does not fully explain our observations that age-related decay is only observed in the adaptation of feedback activity but not in kinematic aftereffects.

Second, feedback policies transforming the sensed state into corrective actions may rely on internal models that are updated through exposure to an alternative environment (Bhushan and Shadmehr, 1999). Lastly, the state estimation following split-belt walking may be inaccurate due to sensory shifts (Vazquez et al., 2015) leading to altered feedback commands even if the feedback policy remains unchanged. In conclusion, the structure of feedback activity post-adaptation reflects the recalibration of the motor system during split-belt walking, suggesting that the feedback loop generating feedback responses is also recalibrated.

Neural mechanisms underlying the adaptation of feedback activity. Feedback and feedforward motor commands might share internal models (Wagner and Smith, 2008; Weiler et al., 2017), which rely on the cerebellum for their adaptation and on cerebral structures for their execution post-adaptation. More specifically, feedforward adaptation and learning of internal models require intact cerebellar function (Martin et al., 1996; Smith and Shadmehr, 2005; Morton and Bastian, 2006). Thus, the cerebellum may also be required for adapting feedback responses. This is supported by the cerebellar dependency on timely recruitment (Herzfeld et al., 2014a) and appropriate magnitude of feedback responses to predictable perturbations (Jacobs and Horak, 2007). Moreover, feedback responses to recover balance are also modulated by prior experience through cortical input (Jacobs and Horak, 2007; Bolton, 2015). Therefore, it is possible that the execution of feedback activity is also influenced by cerebral structures (de Kam et al., 2018), as has been shown in volitional arm control (Pruszynski et al., 2016). Thus, we speculate that the cerebellar and cerebral areas play a role in the adaptation and execution of feedback activity, respectively, but future studies are needed to explicitly address this question.

Age-related decline in the adaptive nature of feedback activity. Aging affects the adaptation of muscle activity after, but not during, split-belt walking, suggesting a possible dissociation between the update of feedback and feedforward activity. Motor learning was affected by age as indicated by the reduced magnitude of aftereffects and less mirroring in feedback activity post-adaptation. It has been suggested that older subjects have noisier sensory information (Konczak et al., 2012), and thus, rely less on sensory input and more on predictive mechanisms (Wolpe et al., 2016). This would explain both the slightly weaker feedback responses upon environmental transitions and reduced recalibration of internal models (i.e., smaller β_M) in older subjects. Despite the age modulation of muscle activity aftereffects, this age dependency is not observed in kinematic aftereffects (Sombric et al., 2017; Bruijn et al., 2012). Perhaps, EMG signals are more sensitive to differences

in internal model recalibration because they are a closer correlate of neural activity than kinematics. Further, the magnitude of muscle patterns during late adaptation, which is mostly feedforward-generated activity, was not affected with age. This dichotomy in age effects on feedback vs. feedforward activity supports that the adaptation of these two processes is partially dissociated (Yousif and Diedrichsen, 2012). We conclude that age-related sensory decline might contribute to motor learning deficits in older adults, which is observed in the adaptation of feedback responses, but not in the steady state motor pattern.

Clinical Implications. Split-belt walking can potentially correct gait asymmetry post-stroke (Reisman et al., 2013), but little is known about the changes in muscle activity that can be expected from this task. Unlike previous work (Dietz et al., 1994; Raja et al., 2013; Ogawa et al., 2014) we provide a systematic characterization of healthy motor patterns during distinct phases of the gait cycle. These normative data allow for the assessment of adaptation deficits post-stroke and early evaluation of the therapeutic limits of split-belt walking. Furthermore, our findings suggest that learning might be limited in patients simply because of their age. Also, inter-subject learning capacities may be better captured through EMG-based measurements than conventional kinematic measurements. Lastly, aftereffects might be more strongly influenced by feedback responses than changes in feedforward circuits, which are arguably the true target of rehabilitation therapies. Thus, long-term rehabilitative potential of split-belt walking may not be well captured by measuring kinematic behavior immediately after adaptation.

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Muscle name	Abbrev.
Tibialis anterior	TA
Peroneus longus	PER
Medial Gastrocnemius	MG
Lateral Gastrocnemius	LG
Soleus	SOL
Biceps Femoris	BF
Semitendinosus	SMT
Semimembranosus	SMB
Rectus Femoris	RF
Vastus Lateralis	VL
Vastus Medialis	VM
Sartorius	HIP
Adductor Magnus	ADM
Gluteus Medius	GLU
Tensor Fasciae Latae	TFL

Table 1. List of recorded muscles & abbreviations.

ID	Sex	Age (y.o.)	Mid speed (m/s)
S01	F	46	0.94
S02	F	51	1.02
S03	F	65	1.08
S04	F	58	0.9
S05	M	57	1.04
S06	M	52	1.05
S07	M	78	0.66
S08	F	52	1.16
S09	M	68	0.85
S10	F	62	0.98
S11	M	75	1.11
S12	M	57	0.99
S13	M	52	1.16
S14	M	64	1.25
S15	M	74	1.11
S16	M	49	1.08
Avg.	10M/6F	60 ± 9.9	1.02 ± 0.14

Table 2. Subject summary. Bottom values indicate mean $\pm$ standard deviation.

Epoch	[FHS-STO] (ms)	swing SLOW (ms)	[SHS-FTO] (ms)	swing FAST (ms)
B	168 ± 12	393 ± 59	166 ± 29	383 ± 43
eA	177 ± 46	314 ± 64	135 ± 34	458 ± 128
lA	176 ± 21	329 ± 38	161 ± 30	473 ± 114
eP	150 ± 35	358 ± 91	188 ± 32	372 ± 69
lP	187 ± 24	405 ± 42	181 ± 33	394 ± 52

Table 3. Mean time elapsed between relevant kinematic events (heel-strikes, toe-offs) during the different epochs of the experiment. Intervals are presented in order of occurrence in the gait cycle, starting at the fast leg's heel-strike (FHS). Median $\pm$ interquartile range values across participants.

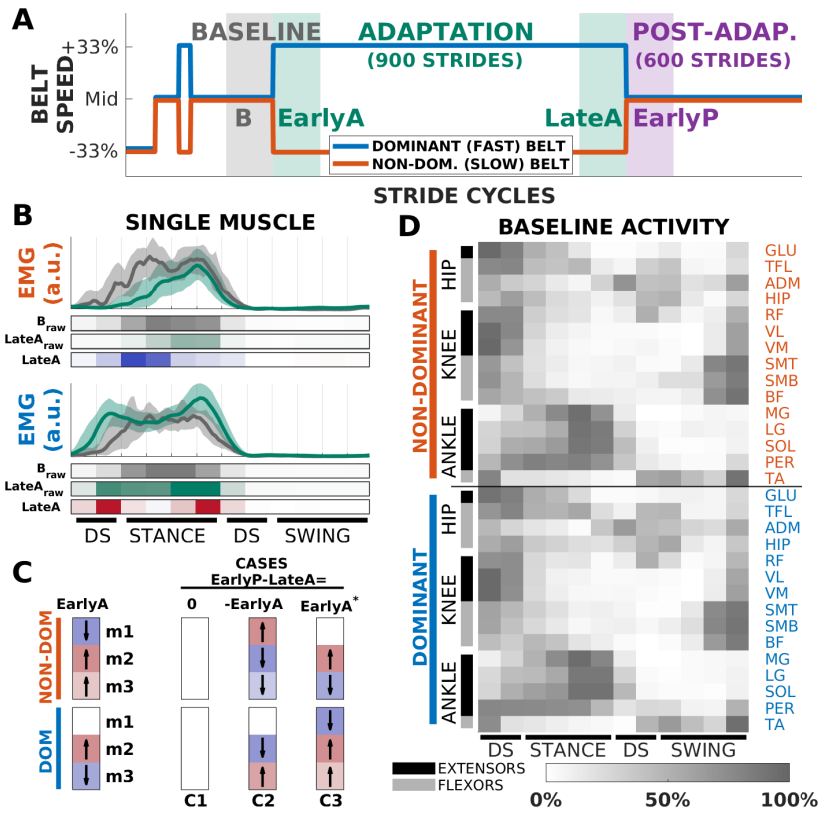

Figure 1

Figure 1 Summary of methods utilized in this study. **(A)** Schedule of belt speeds experienced by all subjects. Shaded areas represent epochs that will be the focus of analysis: Baseline (B), early Adaptation ($EarlyA$), late Adaptation ($LateA$) and early Post-Adaptation ($EarlyP$). **(B)** Sample EMG traces of one muscle (LG) during Baseline (gray) and late Adaptation (green) for a representative subject (S14). Median activity across strides (lines), and the 16-84 percentile range (shaded). Data was processed as described in Methods and further lowpass filtered solely for visualization purposes. Colorbars below the traces represent averaged normalized values during 12 kinematically-aligned phases of the gait cycle (see Methods) for Baseline (B_{raw}, gray), late Adaptation ($LateA_{raw}$, green), and the difference ($LateA = LateA_{raw} - B_{raw}$, red indicates increase, blue decrease). Top panels: data for non-dominant/slow leg. Bottom panels: dominant/fast leg. **(C)** Schematic for possible structure of feedback activity following removal of the perturbation (i.e., $FBK_{split-to-tied} = EarlyP - LateA$). Full description and rationale are presented in the Methods (section 2.4). In brief, One possibility (C1) is that $FBK_{split-to-tied}$ is much smaller than feedforward activity, which carries over from the split to tied condition.

Consequently, Muscle patterns during $LateA$ will be very similar to those during $EarlyP$ (i.e., $EarlyP - LateA = 0$). Another possibility (C2) is that feedback activity is purely environment-dependent. That is, feedback processes generating corrective muscle responses are not adapted during split-belt walking. Therefore, feedback activity is produced solely as a function of the sensed environment. In this case, feedback responses upon removal and introduction of the perturbation will be the numerically opposite from each other (i.e., $FBK_{split-to-tied} = -EarlyA$). Lastly, feedback activity is indicative of the motor system's recalibration during sensorimotor adaptation (C3). In this case, feedback activity post-adaptation would be the mirrored feedback activity during early adaptation (i.e., $FBK_{split-to-tied} = EarlyA^{*}$). This is because subjects adopt the split pattern as their new reference, such as deviations from it are processed as the opposite perturbation to the one originally experienced. **(D)** Summary of muscle activity during Baseline walking for all muscles, averaged as in panel B. Median across subjects. The activity of each muscle is aligned to start at ipsilateral heel-strike. Therefore, each column of muscle activity variables is synchronous for all muscles in the non-dominant (top panel) and dominant (bottom panel) legs separately, but not across legs.

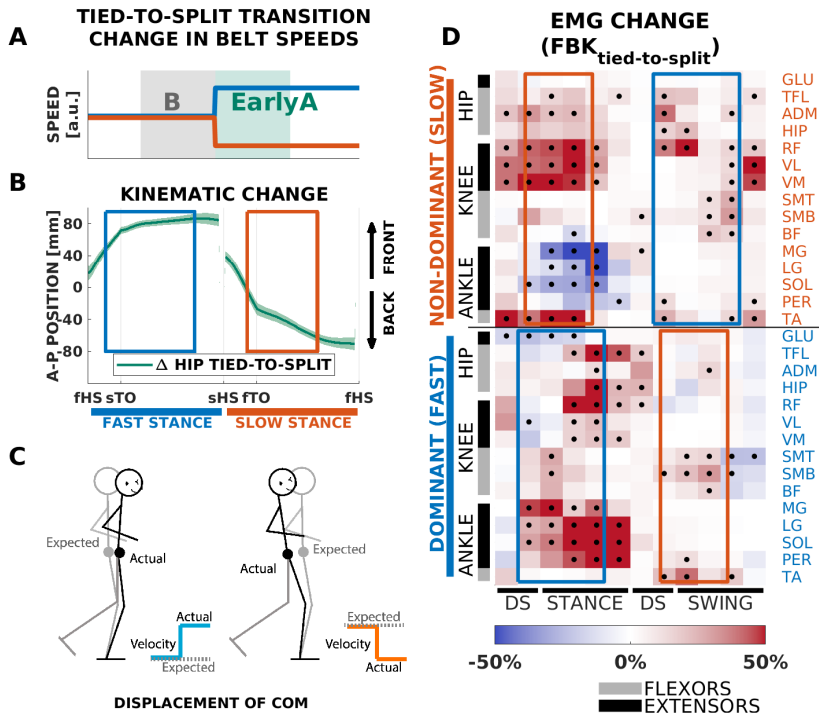

Figure 2. Muscle activity during early Adaptation reveals coordinated feedback-mediated activity to recover balance in response to changes in environmental dynamics. **(A)** Tied-to-split belt-speed transition. **(B)** Relative position of the hip in the anterior-posterior direction, with respect to the (leading) stance ankle during early Adaptation, averaged across subjects. Baseline values are subtracted to illustrate the kinematic changes elicited by walking in the split-belts condition. Positive values represent the hip further ahead from the ankle. Colored rectangles indicate the interval between 100 to 400ms after heel-strike. **(C)** Depiction of postural differences between early Adaptation (black stick figures) and Baseline (gray) behavior. COM data is consistent with subjects 'falling' forward (with respect to prior expectation) when standing on the fast belt, and backwards on the slow leg. **(D)** Modulation of muscle activity during early Adaptation with respect to Baseline (median across subjects). Red colors indicate increases in activity, while blue colors indicate decrease. Dots indicate significant differences (FDR controlled, p-value threshold: $p = 0.041$) (see Methods). Muscle activation variables were displayed starting with the ipsilateral heel-strike. Colored rectangles indicate the same interval as in panel (A), where postural responses are expected to be present. Postural responses to 'falling' can be observed in the data. For example, TA on the slow leg is active during stance, which does not occur during normal walking. This activity is consistent with pulling the COM forward, closer to its expected position.

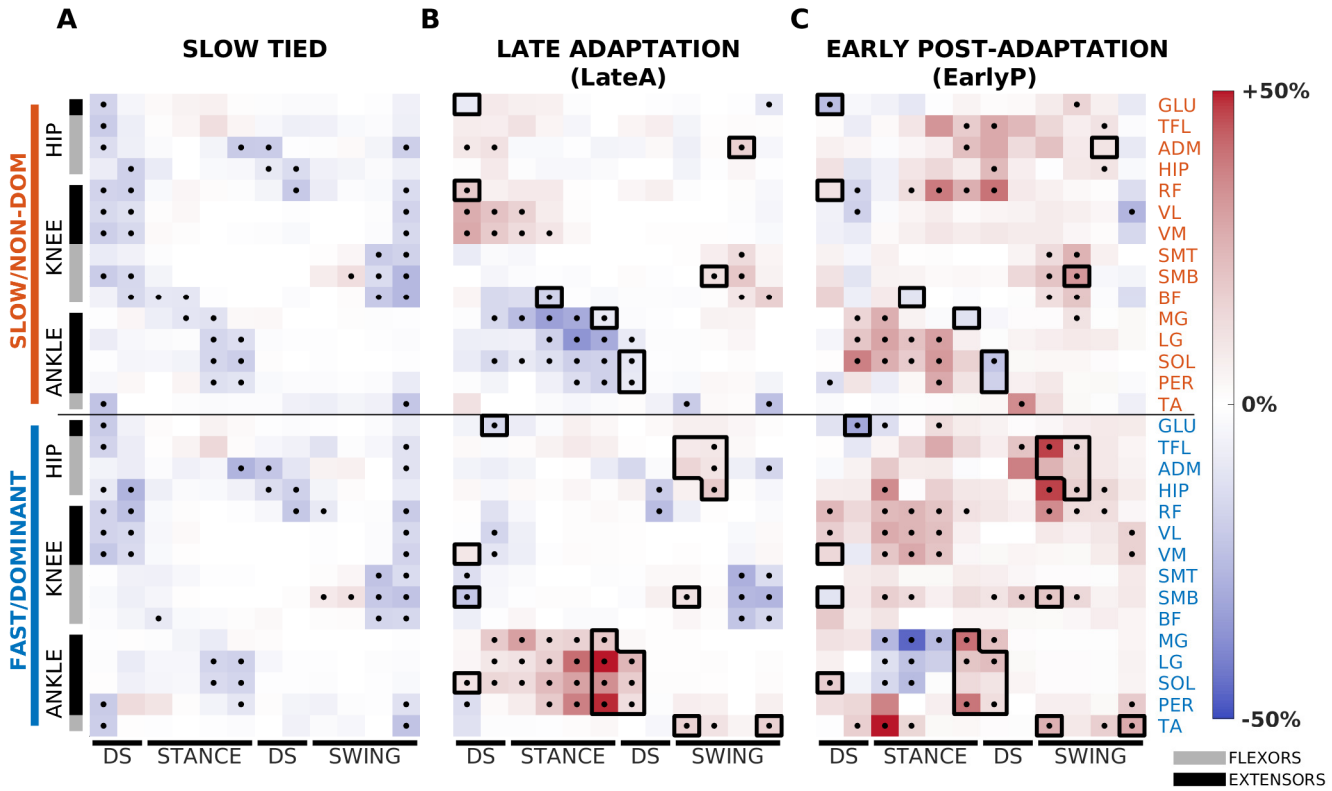

Figure 3. Steady-state muscle activity during slow walking, split-belt walking, and aftereffects. Panels reflect differences in muscle activity the three epochs with respect to the reference (Baseline) condition. Colormap reflects effect size and dots indicate FDR controlled significant differences (see Methods). Muscle activation variables were displayed starting with the ipsilateral heel-strike. **(A)** Muscle activity modulation during slow tied-belts walking. Most muscle-phases show reduction of activity, consistent with a monotonic link between walking speed and muscle activity amplitude. P-value threshold: $p = 0.041$. **(B)** Muscle activity modulation during late Adaptation. Broadly, patterns of activity are anti-symmetric, with groups of muscles increasing activity in one leg and decreasing contralaterally. Those patterns do not match the expectation from simple ipsilateral speed modulation. For example, activity increases in knee muscles on the slow leg (VM, VL, RF, BF, SMT, SMB). P-value threshold: $p = 0.035$. **(C)** Muscle activity modulation during early Post-Adaptation. Few similarities are found between the steady-state activity during late Adaptation and observed aftereffects. Black lines highlight muscle-phases with the same modulation sign (i.e. increase or decrease with respect to Baseline) for both late Adaptation and early Post-Adaptation. We propose these patterns of activity (aftereffects) are generated by a superposition of feedforward- and feedback-generated activity. P-value threshold: $p = 0.035$.

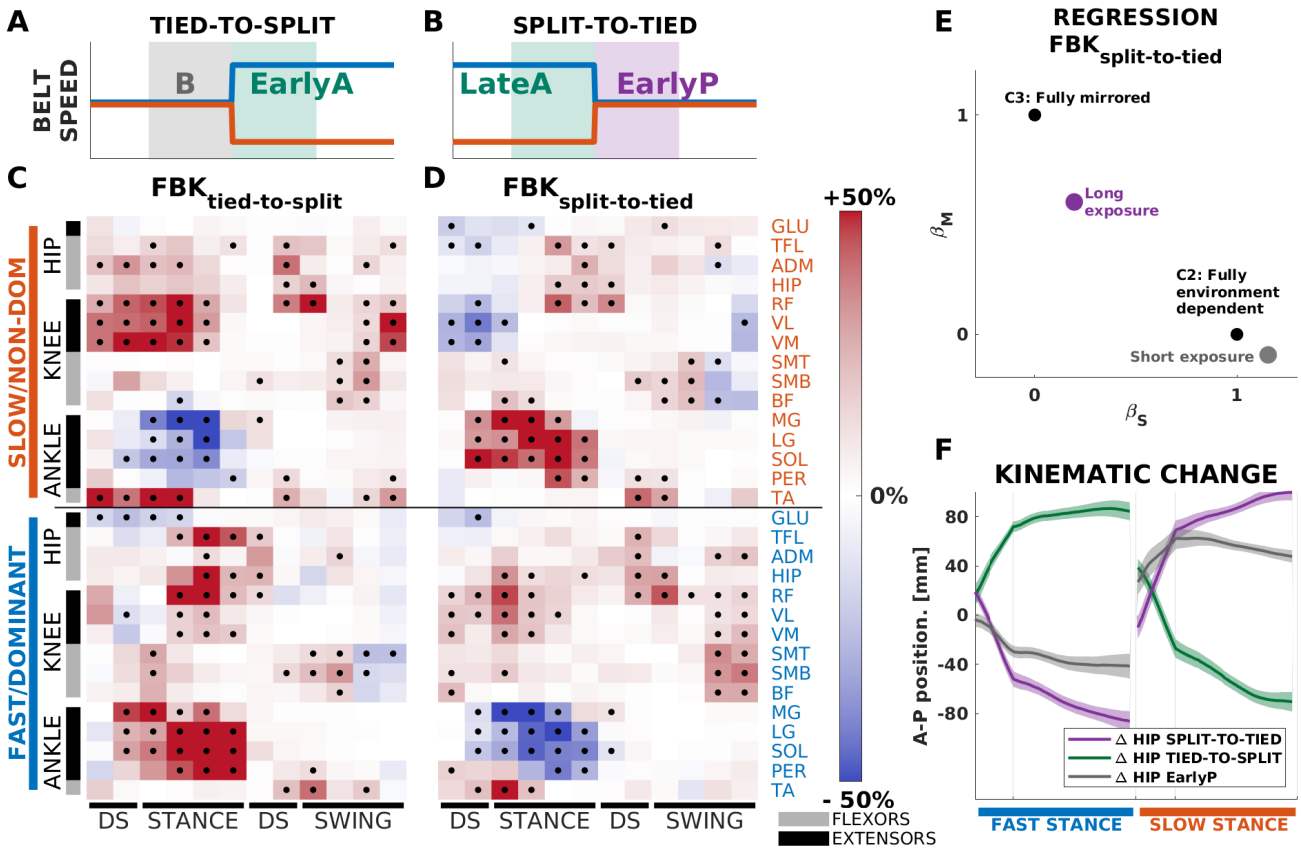

Figure 4. Comparison of changes in muscle activity following two environmental transitions. **(A)** Tied-to-split belt-speed transition. **(B)** Split-to-tied belt-speed transition. **(C)** Tied-to-split changes in EMG data. Same as Figure 2C. P-value threshold: $p = 0.041$. **(D)** Split-to-tied change in EMG data (early Post-Adaptation with respect to late Adaptation). We observe that changes in activity in the fast (slow) leg during this transition resemble those of the slow (fast) leg during the tied-to-split transition (mirroring). For example, TA is not active during stance in steady-state walking (see Figures 1D and 4A,B), but it is active following the tied-to-split transition in the slow leg, and the split-to-tied transition in the fast leg. P-value threshold: $p = 0.035$. **(E)** Quantification of environment-dependent vs. adaptive feedback activity in split-to-tied transitions, after the short exposure (SE, gray) and long exposure (LE, magenta) to the split-belts environment (group averaged data). Black dots represent the results expected from two of the cases discussed in the Methods (C2, C3). **(F)** Changes in hip position (with respect to previous steady-state) after the two transitions (tied-to-split in green, split-to-tied in magenta). Line represents mean across subjects, shaded area is standard error. We observe these the kinematics of these two transitions are approximately mirror images of one another, possibly causing the observed mirroring of EMG responses. For comparison, we also present early Post-Adaptation with respect to Baseline (*EarlyP*, gray).

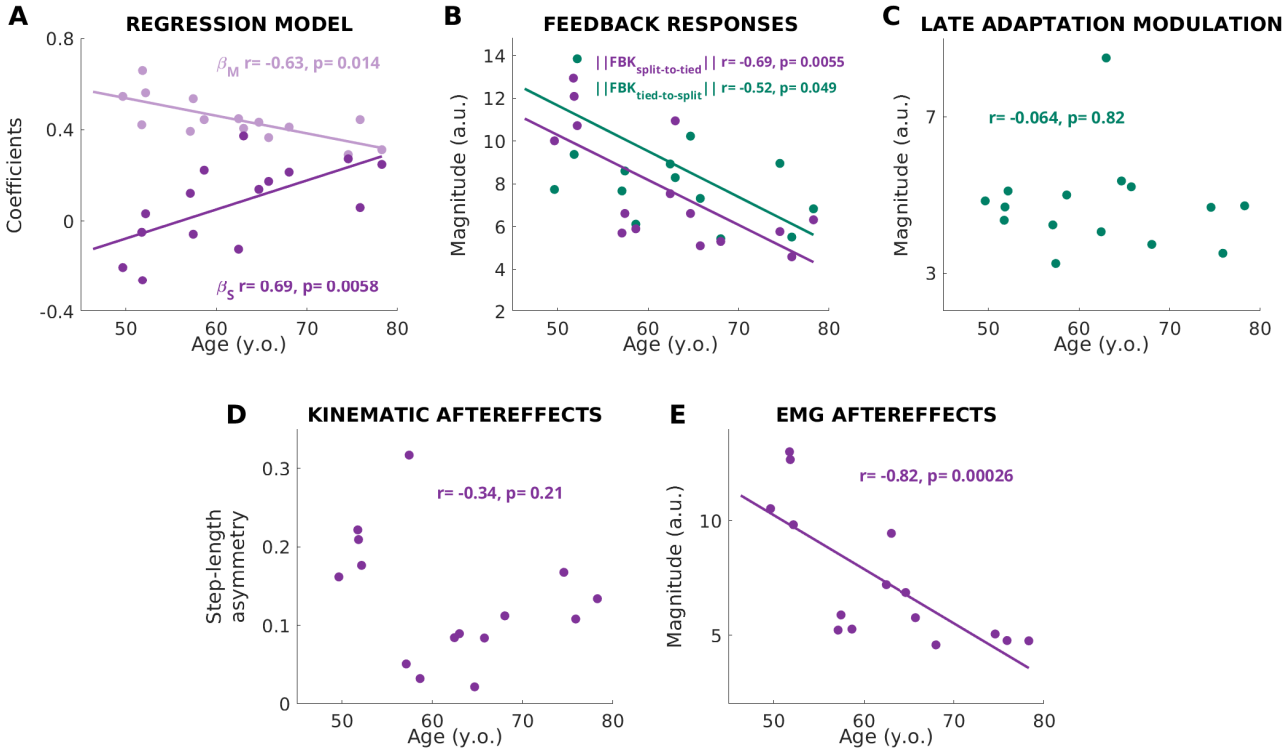

Figure 5. Age modulates EMG-based learning measures, but not kinematic ones. Single dots represent values for one subject. Spearman's correlation coefficients (r) and p-values (p) are presented on the legend. When significant (p<.05) the best line fit of the dependent variable onto age is also presented. **(A)** Regressors from model used to test our hypothesis on feedback responses in muscle activity (dark: β_M, light: β_S). Both regressors are significantly correlated with age. **(B)** Size of muscle activity change following transitions in environmental conditions (feedback responses). Initial feedback responses ($\|FBK_{tied-to-split}\|$) are only weakly correlated with age, but when returning to tied-belts ($\|FBK_{split-to-tied}\|$) there is a significant age effect. **(C)** Size of muscle activity modulation during late Adaptation. (steady-state). No correlation to age was found. This confirms that older subjects are able to modulate muscle activity as much as healthy subjects. **(D)** Step-length asymmetry aftereffects are also not correlated with age. **(E)** Size of muscle activity modulation during early Post-adaptation (aftereffects). Aftereffects are correlated with age. This shows EMG-based measures of learning are more sensitive than kinematic-based ones.

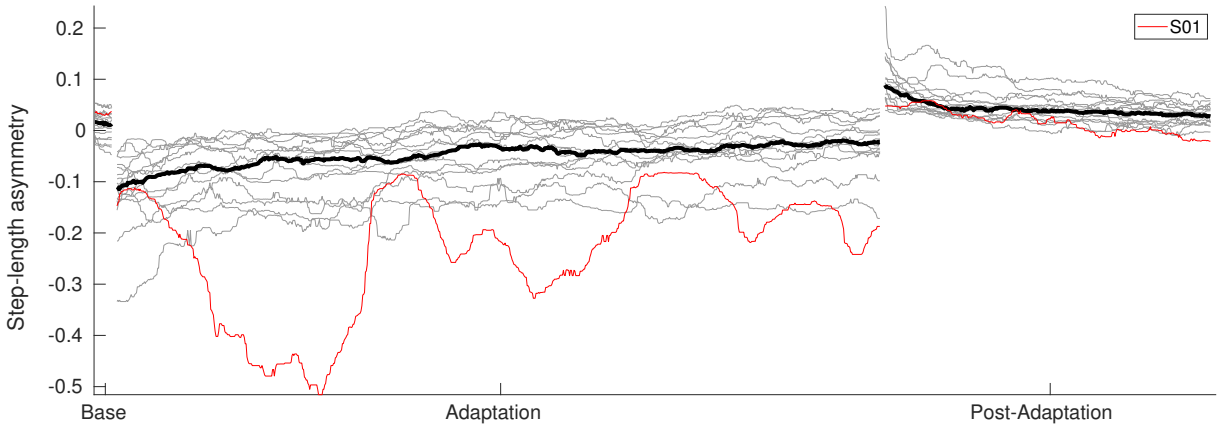

Figure 1-1. S01 is an outlier and did not adapt. **(A)** Step-length asymmetry through Baseline, Adaptation and Post-Adaptation. Data was filtered with a running median filter of width 31 samples. S01 shown in red, other subjects in gray. Group median shown in black.